Experimentally induced Ovulation in the Rhesus Monkey *(Macaca mulatta)*[1]

by

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Dedicated to Kitty Ponse as an expression of our esteem

Growth of ovarian follicles can be induced readily in infantile, prepuberal and adult monkeys by the injection of appropriate gonadotrophic substances. Multiple large follicles result from implants of anterior pituitary tissue, from the injection of unfractionated sheep and pig pituitary extracts as well as their purified follicle stimulating fractions (FSH), and from equine serum gonadotrophin (Allen, 1928; Courrier, Kehl and Raynaud, 1929; Hartman and Squier, 1931; Hisaw, Fevold and Leonard, 1931; Engle, 1933; de Fremery, 1939).

Follicular development with ovulation, however, has been more difficult to achieve. In the studies presented here, an increase in the number and size of growing follicles has followed the use of sheep pituitary FSH, and of both unfractionated and fractionated

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monkey pituitary extracts. Under such stimulation ovaries weighing less than 0.1 to 0.2 gm have been increased within a week to 2 to 4 gm. Ovaries having initially only a few follicles 0.5 to 1 mm in diameter, developed within this period multiple follicles as large as 3 to 8 mm in diameter. If injections were continued to within 24 to 36 hours of removal of the ovaries, the follicles were still healthy, with intact granulosa showing mitoses, and no macrophages in the antrum. Such ovaries were translucent due to the multiplicity of large vesicular structures but, usually, were not cystic, the follicles characteristically not exceeding ovulation size. The granulosa was either compact or showed pre-ovulatory scattering, especially in the cumulus. Invasion of granulosa by blood vessels and theca cells was not usually observed following moderate doses of purified FSH. When high doses, or preparations of FSH contaminated heavily the interstitial cell stimulating hormone (ICSH), were given, epithelialization of the membrana granulosa and of the theca interna was observed. In such instances luteinized structures were sometimes formed, resembling corpora lutea, but with enclosed ova. Administration of supplementary pituitary interstitial cell stimulating hormone (ICSH), or human chorionic gonadotrophin (HCG) increased the extend of thecal luteinization. In common with the experience of other workers in this field, it has proven difficult to determine the effective conditions for induction of ovulation and normal corpus luteum formation (Engle, 1934; Hisaw, Greep, Fevold, 1935; Hartman, 1938 and 1942, van Wagenen and Simpson, 1957).

In the effort to establish the conditions necessary for ovulation, the efficacy of follicle stimulating agents from different pituitary sources was investigated, as was the optimum dose levels and the duration of treatment required to produce follicles of mature size in monkeys of different ages. It was also necessary to determine the requirement for a luteinizing agent, the proper dosage, and the time of administration in relation to follicular growth. In the adult animal it was necessary to relate these factors to the time in the menstrual cycle.

Conditions which proved effective in producing ovulation in adult and preadolescent monkeys are recorded in Table I. The first experiments listed are instances of ovulation after stimulation

of the ovary by monkey anterior pituitary preparations.[1] Four mature monkeys received injections of pituitary fractions beginning the 5th or 6th day of the menstrual cycle. The injections of pituitary substance were continued for 8 or 9 days and were supplemented during the last 4 days of the period by high doses of HCG.[2] Three of the four animals (Mm 884, 878, 812) so treated ovulated. In the one which failed to ovulate the dose of the pituitary preparation was somewhat lower and that of HCG was only 1/6 the dose which resulted in ovulation in the other three. Normal cyclic ovulation of a single ovum proceeded in this monkey without interference from the excessive follicular stimulation. The rhesus monkey characteristically matures one ovum each menstrual cycle, as does the human female, instances of twinning with non-identical offspring occurring in no larger proportion of normal births than in the human.[3] Experimentally induced ovulation, on the other hand, was characteristically multiple. Therefore, only those instances in which multiple corpora lutea, and multiple stigmata were present, were considered to be the result of treatment in the adult macaque.

Monkey Mm 812 and Mm 878 received comparable dosages of the two hormones, monkey anterior pituitary 40% alcohol preparation and chorionic gonadotrophin, beginning on the fifth day of the menstrual cycle. Mm 812 (9 years, 6,850 gm) received 2.5 mg of the pituitary preparation twice daily, 5 days, subcutaneously, after which the dose was reduced to 1.5 mg given mixed with 600 IU HCG twice daily, 4 days. The left ovary was removed on the 15th day of the menstrual cycle, the 2nd day after injections

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[1] Pituitaries of *Macaca mulatta* were obtained from the Cutter Laboratories, Berkeley, through the courtesy of Walter E. Ward, Donald Trotter and Donald H. Wonder, and from the Statens Seruminstitut, Copenhagen, through the courtesy of Preben and Herdis von Magnus. The anterior lobes were dissected within 2 hours after death of the animals and frozen immediately. The glands were counted, pooled, weighed and lyophilized. Forty percent ethyl alcohol extracts were made and the soluble material was lyophilized. The potency of the preparation in terms of units of FSH and ICSH (minimal dose for reinstatement of follicular growth, or minimal dose effective in repair of the interstitial cells in the immature hypophysectomized rat) varied from 0.4 mg to 0.75 mg.

[2] The human chorionic gonadotrophin was kindly supplied by Parke, Davis and Company through the courtesy of Daniel A. McGinty.

[3] Among 358 monkey pregnancies recorded in the colony in the Yale Department of Obstetrics, non-identical twins occurred only once.

ceased. It weighed 2.56 gm and showed three open stigmata.[1] The corpora lutea were variable in degree of development and were estimated to be from one to four days old [2] (Fig. 1). This would indicate that ovulation began soon after administration of the high dose of luteinizing agent. On removal of the remaining ovary seven days later (22nd day of the cycle) the corpora lutea were found to be well consolidated, conforming in structure to corpora lutea 10 to 12 days old (Fig. 2). This ovary was embedded in adhesions which had resulted from the extensive disruption of the ovarian surface incident to multiple ovulation. This made it impossible to dissect the ovary accurately for weighing, and interfered with identification of stigmata, but it could be determined that at least five of the large corpora lutea had expelled their ova. The uterus held an actively secreting endometrium indicating the presence of functional lutein tissue.

Mm 878 (5 years, 5,095 gm) received 2.5 mg of the pituitary preparation twice daily, 4 days, subcutaneously, after which it was reduced to 1.5 mg and given mixed with 600 IU HCG twice daily for 4 days; 50 mg of lactogenic hormone was injected daily for 3 days, the period overlapping the two last days gonadotrophin was given. The ovarian response was examined on the next day, the 14th day of the menstrual cycle. At laparotomy five sites of recent ovulation in each ovary presented the appearance of oozing craters, 3 to 4 mm in diameter, rimmed with the everted newly luteinized follicle walls, only one of which is shown in the photomicrograph (Fig. 3). The right ovary removed at this time

[1] The term stigma has long been used to indicate the break in the follicular wall at the surface of the ovary through which the ovum has been expelled. Immediately after ovulation the stigma appears as an irregular tear from which follicular fluid exudes. As the walls of the follicle become luteinized a plug of lutein tissue usually forms which extends as a tuft from the surface of the ovary, later becoming vascularized. When there is a minimum of pressure within, the edges of the torn follicle wall may approximate and the opening close with cicatricial tissue within a few days. When extruding luteal tissue is present it regresses with the aging of the corpus luteum, remaining as a fibrotic tuft for several menstrual cycles. With increased pressure, such as accompanies multiple ovulation, the torn follicle walls evert and the lutein tissue grows out over the surface of the ovary.

[2] The ages of the corpora lutea were determined by comparison with the structure of those in a series of ovaries removed from normal monkeys on known days of the cycle; also by comparison with Corner's description and illustrations of the development and regression of the corpus luteum in the macaque (1945).

weighed 1.42 gm. Six days later, on the 20th day of the menstrual cycle, the contralateral ovary (left) contained eight corpora lutea, 5 of which had identifiable ovulation stigmata. The craters by this time were filled with luteal tissue and the corpora lutea had become solid structures, but the points of rupture were still indicated by mushroom-like protruberances from the surface of the ovary (Fig. 4). From their structure, the ages of these corpora lutea were estimated to be 10 to 11 days. The second ovary weighed 0.69 gm, less than half the first, due to discharge and absorption of follicular fluid.

Multiple ovulation was also induced in the adult monkey Mm 884 (6½ years; 5,180 gm). The pituitary fraction made from pooled anterior lobes of macaque monkeys was injected at 2.5 mg doses, twice daily subcutaneously, for five days, beginning the sixth day of the menstrual cycle. During the ensuing four days the dose of the pituitary fraction was reduced to 1.5 mg and injected twice daily mixed with 600 IU of HCG. The left ovary removed 36 hours after the last injection, on the 16th day of the cycle, weighed 3.88 gm. It contained, in addition to large follicles, 12 corpora lutea. A rupture point was identified in each corpus luteum. Four of the corpora lutea and two open stigmata are shown in Fig. 5. The ages of the corpora lutea were less than 6 days, probably between 2 and 4 days, indicating that here, too, ovulation had followed soon after the beginning of HCG administration. Figure 6 shows in more detail the wall of a corpus luteum.

Six months later an exact duplication of this course of treatment was carried out in this animal. The 2nd ovary, removed nine days after the end of this injection series, weighed 1.42 gm. Multiple follicles had developed and many were luteinized, resembling structurally 10 to 12 day corpora lutea. Though they approached the surface of the ovary no stigmata were present. Stigmata from interim cycles were present and the one for the current cycle was recognizable, indicating that treatment had not interferred with normal ovulation.

A prepuberal monkey (Mm 887, 1 yr 9 mo, 3,120 gm), also, was induced to ovulate by injection of the monkey pituitary preparation supplemented by HCG, under conditions of treatment similar to those successful in the adults. This animal had shown no menarchal activity judged from lack of vaginal desquamation, sex skin

swelling or breast development. The 40% alcohol preparation made from monkey anterior pituitaries was injected in doses of 5 mg, twice daily, subcutaneously, for 6 days, followed by half this dose mixed with 800 IU HCG, given twice daily by the same route for the next 4 days. Laparotomy on the 2nd day after the last injection disclosed that one ovulation site was present in the left gonad beside several corpora lutea without surface defects indicating ovulation. Examination of the ovaries 7 days later showed multiple corpora lutea, but ovulation could be established to have occurred from only one corpus luteum contrasting with the multiple ovulation characteristic of the adult response (left ovary Fig. 12, arrow).

Ovulation was also successfully induced in one adult monkey by injection of monkey anterior pituitary material without the high dose of chorionic supplement. Mm 955 (7 years, 5,400 gm) was injected from the 5th day of the menstrual cycle, twice daily subcutaneously, with 5 mg of a 40% alcohol preparation for 9 days.[1] Both ovaries were removed the 4th day after termination of injection. They weighed approximately the same, 0.45 gm. Five corpora lutea, estimated to be less than 6 days old, were present. Coagulated fluid still clung to the stigmata of the corpora lutea (Fig. 7). The histological detail of a stigma from this ovary is shown in Fig. 8. Examination of the ovaries showed a few additional luteinized bodies from which the ova had not been discharged.

Although both adult and immature monkeys ovulated soon after addition of the luteinizing agent (HCG), it must be kept in mind that ovulation can occur without high terminal supplementary doses of this luteinizing agent. Chorionic gonadotrophin may not be effective in initiation of ovulation solely through its ability to epithelialize, or luteinize, the theca interna. The increased vascularity resulting from the chorionic supplement should be con-

[1] The dose of monkey pituitary preparation was somewhat higher in this animal (Mm 955) than in those listed in the table which had ovulated after receiving the chorionic supplement. In the five other adult monkeys which received monkey pituitary preparation without HGG supplement the absence of ovulation in two could be attributed to initiation of injection too late in the cycle, days 9 and 15 respectively. Normal ovulation, judged by the presence of a single young corpus luteum with open stigma, had occurred in these animals uninhibited by the excessive follicular growth induced. In the remaining two monkeys of this group the negative results might be attributed to the removal of the ovary too early in the cycle (day 12); collapsed follicles were present, but it was too early to be certain of corpus luteum formation.

sidered because this hormone is known to increase the blood supply to the ovary. It is known also that chorionic hormone, in some species at least, augments follicular response to simultaneously injected pituitary FSH. Furthermore the participation of the recipients's own pituitary must be kept in mind, as stimulation of the pituitary by the chorionic hormone modifies the physiological action in normal and hypophysectomized animals.

The primate source of the pituitary preparations used in these studies may have been an important factor in determining their effectiveness in the induction of ovulation. The recent success in promoting growth and metabolic changes in the monkey by injection of primate pituitary growth hormone, a result conspicuously lacking after injection of growth hormone from beef anterior pituitary, has been interpreted as due to species specificity of the hormone (Knobil and Greep, 1956; Knobil, Morse and Greep, 1956). However, it must be remembered that induction of ovulation in the rhesus monkey has been reported by several workers following injection of gonadotrophins from heterologous species.

It has been possible also in this study to induce ovulation, both in prepuberal and adult rhesus monkeys, by injection of sheep pituitary gonadotrophins. An instance of successfully induced ovulation in the adult following injection of sheep pituitary FSH supplemented by HCG, is illustrated (Figs. 9, 10, 11). Mm 952 (7+ years, 6,800 gm) was injected from the 5th day of the menstrual cycle for 9 days with sheep pituitary FSH [1] in doses of 10 mg given twice daily for 5 days, followed by doses of 5 mg mixed with 800 IU HCG given twice daily for 4 days, all dosage being subcutaneous as in previous experiments. (Total FSH, 1800 RU, total HCG 6400 IU.) The ovaries were not removed until 5 days after termination of injection (18th day of the menstrual cycle) in order to give adequate time for ovulation and development of well formed corpora lutea. The two ovaries of this animal showed 1 and 3 stigmata, respectively. Well formed corpora lutea were present, estimated to be older than 6 days, again indicating that ovulation had followed immediately after onset of administration

[1] The sheep pituitary FSH and ICSH were prepared by ammonium sulphate and isoelectric fractionation of 40% alcohol extracts of acetone dried whole pituitaries.

of HCG. Photomicrographs are shown of sections of both ovaries in order to show that ovulation was multiple (Figs. 9 and 10). Details of the stigma in the left ovary are shown in Fig. 11.

Ovulation in a premenarchal monkey following injection of sheep pituitary FSH supplemented terminally by a large dose of luteinizing agent is exemplified by Mm 723 (1½ yr, 2040 gm). In this animal the pituitary luteinizing agent (ICSH) [1] was injected. The left ovary was removed at laparotomy on the 9th day after preliminary treatment with high dosage of FSH (50 mg twice daily for 8 days given subcutaneously). Ovulation was in progress from two follicles, one of which is shown in Fig. 13. Injection of FSH was continued at doses of 25 mg daily given subcutaneously for 4 more days and ICSH was given simultaneously once daily intraperitoneally in 10 mg doses. On the 13th day a single large dose of ICSH, 55 mg, was given intraperitoneally. A total of 4125 RU of FSH and 3600 RU of ICSH was injected. The right ovary, removed 48 hours after the terminal large dose of ICSH, contained 3 young corpora lutea with open stigmata. Details of the wall of one of the corpora lutea are shown in Fig. 14.

In yet another premenarchal monkey ovulation followed injection of sheep FSH, supplemented terminally by the pituitary luteinizing agent, ICSH, Mm 816 (1½ yr, 2,900 gm). At onset, this animal showed minimal sex skin swelling and no perianal swelling. The immature condition of the control ovary is shown in Fig. 15 (Left ovary 0.19 gm). A sheep pituitary preparation, predominantly follicle stimulating, was injected in 20 mg doses, twice daily, subcutaneously, for 10 days, totalling 2000 RU FSH. During the last 4 days the second sheep pituitary preparation, purified ICSH, was injected in doses of 25 mg twice daily, intraperitoneally, totalling 13,000 RU. During the 2 days between termination of injections of the gonadotrophins and autopsy, 30 mg of lactogenic hormone were injected twice daily subcutaneously, totalling 1500 IU.[2] The ovary removed the day after termination of injections (right, 0.91 gm) contained 10 large luteinized bodies, three of which are shown in Fig. 16. One of

[1] See note on page 813.

[2] No effect of lactogenic hormone was detected, either on the occurrence of ovulation or the formation of corpora lutea, in the two monkeys in which it was injected, Mm 878 and 816.

these had ovulated, a well formed stigma being present, shown in another level of the ovary in Fig. 17.

The doses of sheep pituitary FSH which successfully resulted in ovulation were large compared with those of monkey pituitary preparations. However, follicular growth did result in both adult and immature monkeys from adequate dosage of these sheep preparations, and it should be stressed that luteinization occurred on supplementation with luteinizing fractions either of pituitary or placental origin, and ovulation did result in some individuals, both adult and immature. Optimum conditions for induction of ovulation with heterologous pituitary hormones have not been determined. Under the conditions of injection used, false corpora lutea with enclosed ova resulted in many individuals, and frequently false corpora were observed scattered among corpora lutea of ovulation. With neither homologous nor heterologous gonadotrophins have the conditions been determined for induction of the normal number of ovulations typical of the species. Multiple ovulation has characterized the response of the monkey, just as superovulation has been characteristic of the response of other species to exogenous gonadotrophins.

The ovulations obtained bore no relation to the breeding season. Although these monkeys can ovulate and conceive throughout the year, it is generally believed that they breed more readily between September and May, in the northern hemisphere. Months in which ovulation was induced, in these studies, were: March— Mm 955, Mm 952; June— Mm 884 L; July— Mm 878; October— Mm 884 R; November— Mm 812.

Summary

Ovulation has been induced in adult monkeys by injection of extracts of monkey anterior pituitaries supplemented terminally by human chorionic gonadotrophin. Ovulation followed immediately after the luteinizing supplement was added. The chorionic hormone though an important adjunct was not essential inasmuch as ovulation was induced by pituitary preparations without this supplement.

Although ovulation was more easily induced after injection of homologous pituitary material, sheep pituitary follicle stimulating

fractions, supplemented either by human chorionic hormone or pituitary interstitial cell stimulating hormone, were also effective in both adult and prepuberal monkeys.

In adult monkeys, ovulation induced by injection of gonadotrophins characteristically occurred from several follicles. This multiplicity of ovulation sites was useful in distinguishing between induced and normal cyclic ovulation. In the prepuberal monkey following similar treatment a single ovulation was significant.

EXPLANATION OF PLATES

Plate I

Photomicrographs of ovaries of adult macaques treated with homologous anterior pituitary preparations. The animals in Figs. 1 to 6 had received supplementary treatment with human chorionic gonadotrophin (HCG); those in Figs. 7 and 8 no supplement. Hematoxylin and eosin stain.

Fig. 1. Mm 812, left ovary removed on the 15th day of menstrual cycle, 2nd day post-injection, showing one of the three stigmata of young corpora lutea (arrow). × 10

Fig. 2. Mm 812, right ovary, 22nd day, 9th day post-injection, further development of corpora lutea; section shows one of the five stigmata, with protruding lutein tissue (ovary partially imbedded in adhesions). × 20

Fig. 3. Mm 878, right ovary, 14th day, 2nd day post-injection, showing one stigma of the five ovulated follicles. × 10

Fig. 4. Mm 878, left ovary, 20th day, 8th post-injection, showing three of the five corpora lutea, 10 to 12 days old, all with widely exposed luteal surfaces and outflowing tissue. × 20

Fig. 5. Mm 884, left ovary, 16th day post-injection, 2 of 12 corpora lutea present are shown still open and with exuding sanguinous follicular fluid. × 15

Fig. 6. Mm 884, same ovary as in Fig. 5, showing details of wall of corpus luteum. × 125

Fig. 7. Mm 995, left ovary, removed on 17th day, 4th day post-injection, showing the open stigma of one of the four corpora lutea. Multiple ovulation occurred without chorionic supplement. × 13

Fig. 8. Mm 955, right ovary, removed same day as left, showing detail of another stigma through which detached islands of mural tissue flow. × 125

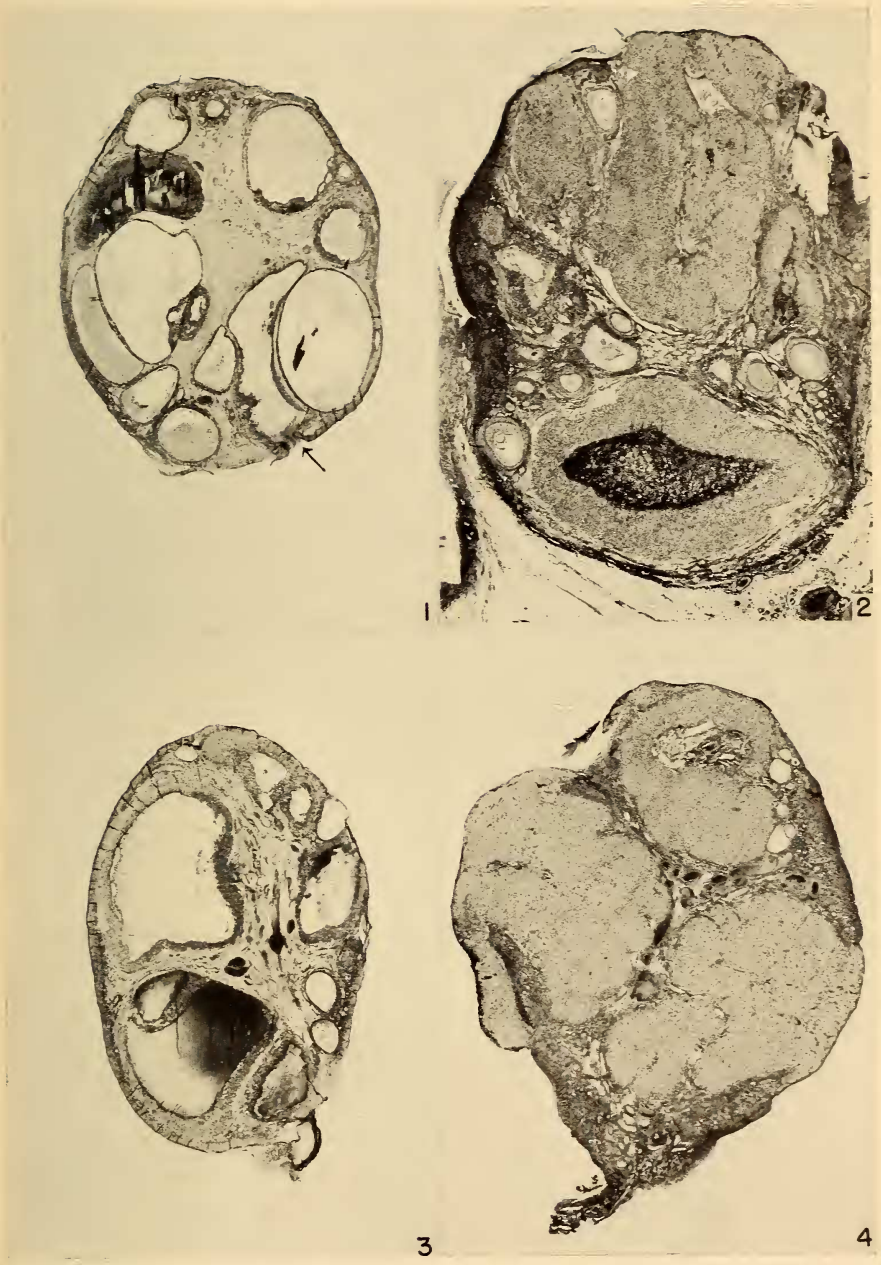
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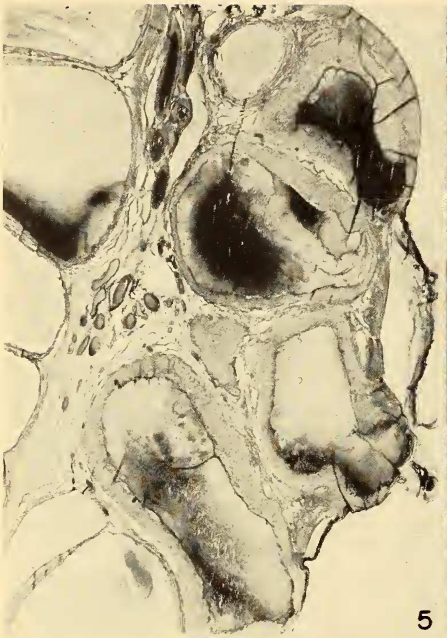
PLATE II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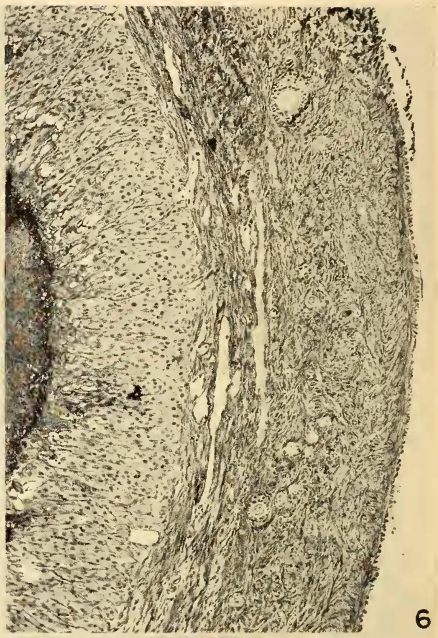
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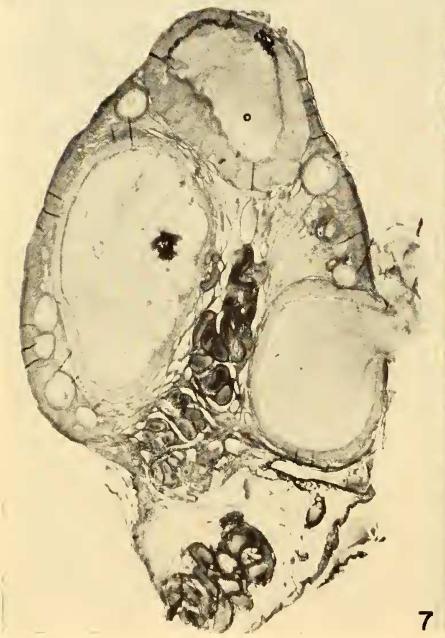
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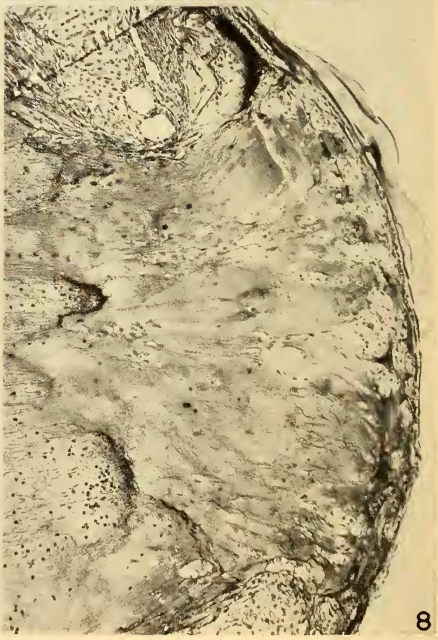
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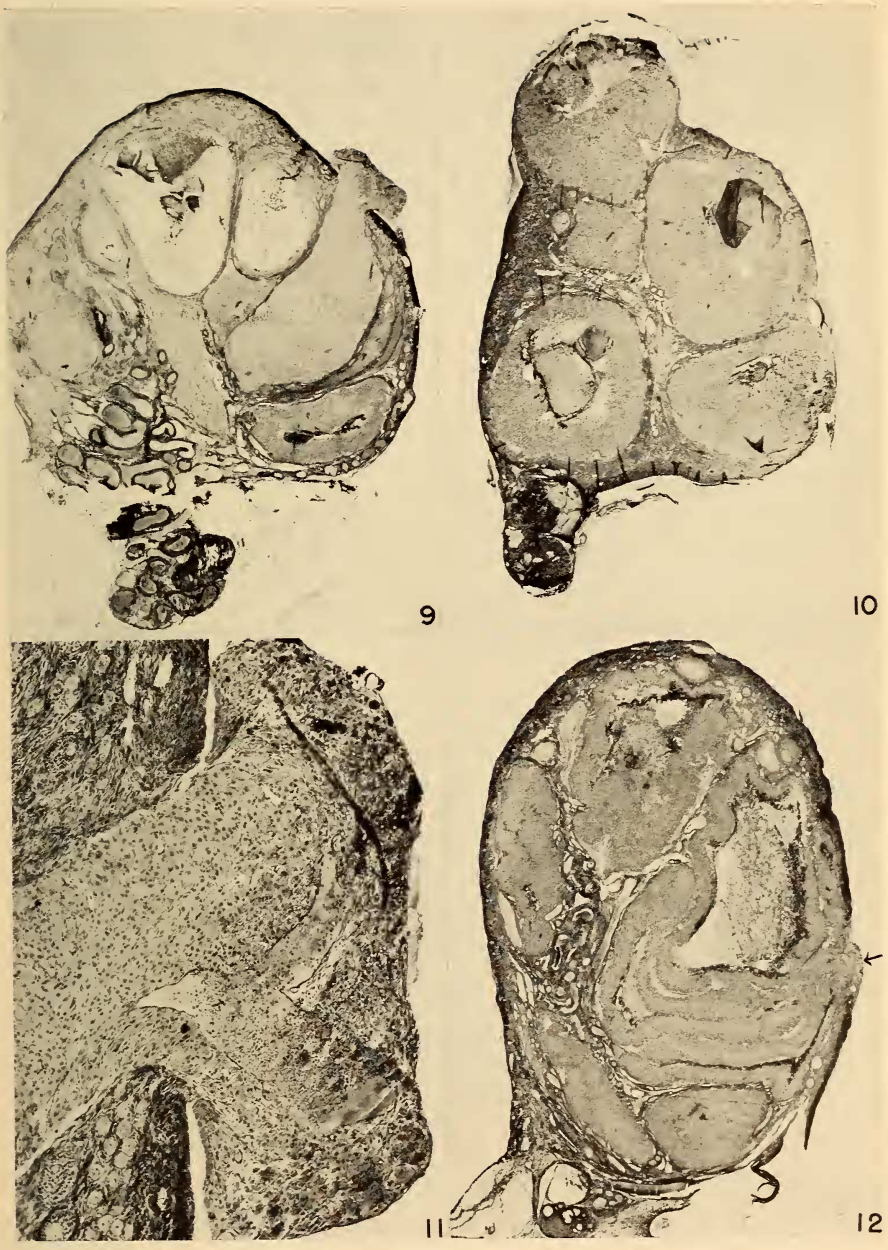
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